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(54) CELL CULTURE APPARATUS

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DESCRIPTION

1. TITLE OF THE INVENTION

CELL CULTURE APPARATUS

2. CLAIMS FOR THE PATENT

A cell culture apparatus configured to supply a culture medium under pressure into a plurality of hollow fibers having

selective permeability to permeate a part of the culture medium into an outer surface side of the hollow fibers and to carry out cell culture in a cell culture space formed in the outer surface area, the apparatus comprising:

a culture-medium-circulating system circulating the culture medium supplied to the hollow fibers under pressure; and

a produced-liquid-circulating system circulating a culture medium containing a low-molecular-weight substance and a high-molecular-weight useful substance produced in the cell culture space through a membrane separation device to separate the low-molecular-weight substance; and

further comprising

pressure-adjusting means to keep a balance of flow rates between the culture-medium-circulating system and the produced-liquid-circulating system.

3. DETAILED DESCRIPTION OF THE INVENTION

[INDUSTRIAL APPLICATION FIELD]

The present invention relates to a cell culture apparatus, and, particularly, to a hollow-fiber-type cell culture apparatus which is optimal for performing continuous cell culture at high density, the apparatus achieving uniformity of the environment in a system by sophisticatedly removing unwanted substances which are produced as a byproduct while efficiently supplying nutrients to cells and enriching a produced useful substance.

[CONVENTIONAL ART]

Cell mass culture at high density is an indispensable technique to achieve industrial-scale production of a useful

physiologically active substance such as a monoclonal antibody and a lymphokine.

Classical cell culture has been conducted at a laboratory level by using a culture bottle, etc. However, if it is possible to perform cell culture at high density and with a large amount, it will be industrially advantageous. From this viewpoint, various methods for cell mass culture and apparatuses therefor have been developed.

The culture types of these methods and apparatuses are largely divided into floating (non-adherent) cell culture and adherent (wall-adherent) cell culture depending on the property of cultured cells. For the former floating cell culture, a key point is to maintain a suspension condition of cells and to achieve efficient substance exchange. In contrast, for the adherent cell culture, a key point is to increase the number of adherent cells per unit volume and to efficiently achieve exchange of substances such as a nutrient, a waste product, and gas.

Even if either type of above culture is adopted, the substance exchange remains a key point. Accordingly, cell culture utilizing a hollow fiber or capillary having a selective-separation property has received an attention. For example, the substance exchange which is carried out by using the hollow fiber resembles phenomena that cells attain nutrients via blood vessels from blood in vivo and the cells excrete metabolites. The above substance exchange is considered extremely promising as a cell culture method in vitro, and a variety of culture apparatuses has been developed. Among them,

various improvements have been progressing which include a method for proliferating cells by having the cells attach to the outer surface of hollow fibers by passing a liquid culture medium (culture medium) through the hollow fibers, and which actively carrying out gas exchange of cells present in a liquid culture medium or the outer surface of hollow fibers by flowing gas including oxygen into the hollow fibers. As a cell culture technique using these hollow fibers, techniques of Japanese Patent Application Laid-Open Publication No. 49-41579, Japanese Patent Application Laid-Open Publication No. 50-36684, Japanese Patent Application Laid-Open Publication No. 52-125688, Japanese Patent Application Laid-Open Publication No. 59-175077, Japanese Patent Application Laid-Open Publication No. 61-25477, Japanese Examined Patent Application Publication No. 62-500356, and Japanese Examined Patent Application Publication No. 62-500357 have been disclosed until now.

[PROBLEMS TO BE SOLVED BY THE INVENTION]

Examples of a restriction factor for high density culture of cells include accumulation of growth-inhibiting substances in a system (e.g., metabolites such as lactic acid and an ammonium ion), depletion of essential nutritional sources (e.g., glucose, essential amino acids, vitamins), depletion of dissolved oxygen content, and furthermore a rapid decrease in a pH.

Conventional hollow-fiber-type cell culture apparatuses have been developed to completely eliminate such a restriction factor. However, there has been a situation that the apparatus cannot be said to be an established technique for culturing cells at high density. The actual situation has been that the environment in a

culture apparatus, in particular, does not become completely uniform and cell growth is partially insufficient, which results in a situation that the cell density of the entire culture apparatus fails to increase as expected.

The present invention has been developed to solve such technical problems. It is an object of the present invention to keep the environment in a cell culture apparatus as uniform as possible, thereby providing a hollow-fiber-type cell culture apparatus capable of culturing cells at high density.

[MEANS FOR SOLVING THE PROBLEMS]

The present invention which has achieved the above object is a cell culture apparatus configured to supply a culture medium under pressure into a plurality of hollow fibers having selective permeability to permeate a part of the culture medium into an outer surface side of the hollow fibers and to carry out cell culture in a cell culture space formed in the outer surface area, the apparatus comprising:

a culture-medium-circulating system circulating the culture medium supplied to the hollow fibers under pressure; and

a produced-liquid-circulating system circulating a culture medium containing a low-molecular-weight substance and a high-molecular-weight useful substance produced in the cell culture space through a membrane separation device to separate the low-molecular-weight substance; and

further comprising

pressure-adjusting means to keep a balance of flow rates between the culture-medium-circulating system and the produced-liquid circulating system.

[OPERATION]

In the present invention, a basic configuration is to prevent depletion of nutritional sources and dissolved oxygen, etc., in a cell culture space by supplying, under pressure, a culture medium into hollow fibers. In this case, by adjusting beforehand compositions of the nutritional sources to be supplied into the hollow fibers and a dissolved oxygen content, etc., the supply of a culture medium at an optimal culture condition becomes possible.

It is possible to uniformly supply the culture medium throughout the entire cell culture space by employing a configuration for a culture-medium-circulating system circulating a culture medium supplied into hollow fibers under pressure. Also, in order to more effectively achieve this object, it is recommended to provide the foregoing culture-medium-circulating system with a gas exchanger, preferably a hollow-fiber-type oxygenator to control a dissolved oxygen content or a dissolved carbon dioxide content, and in addition, to install a line to supply a fresh culture medium or a base to supplement the culture medium that has permeated through the above hollow fibers and to adjust the pH.

It is noted that the types of cell in the foregoing cell culture space are not limited. For adherent cells, examples thereof can include a method for adhering cells onto the outer surface of hollow fibers and a method for encapsulating cells into a macromolecular gel matrix such as collagen (Japanese Patent Application No. 62-65363). On the other hand, for floating cells, examples thereof can include a method for

encapsulating cells into the above macromolecular matrix, and a method for culturing cells in a floating condition in liquid.

The culture medium in the cell culture space is fed to a membrane separation device in a condition including produced low-molecular-weight unwanted substances and a high-molecular-weight useful substance. The culture medium that has been fed to the membrane separation device is made to circulate again in the cell culture space after removal of the low-molecular-weight unwanted substances that become a growth-inhibiting factor such as lactic acid and an ammonium ion as cellular metabolites (a produced-liquid-circulating system). It is noted that with regard to this circulating culture medium, the pH and a dissolved oxygen content are adjusted in the produced-liquid-circulating system.

In such a manner, circulation of the culture medium in the cell culture space through the membrane separation device allows cellular metabolites to be quickly removed from the vicinity of the cells and allows the environment in the cell culture space to be uniform. Also, this can prevent a partial or total rapid decrease in the pH due to accumulation of the metabolites from occurring in the cell culture space. Furthermore, removal of the low-molecular-weight unwanted substances by selectively separating them by using the membrane separation device enables the concentration of the growth-inhibiting substances in the culture medium to decrease and enables the high-molecular-weight useful substance which the cells produce to be enriched. Then, by withdrawing the culture medium in which the high-molecular-weight useful substance is enriched with a certain flow rate to

the outside system, and in addition, by that the transportation of the culture medium in the cell culture apparatus is stabilized by controlling the flow rate of the culture medium permeated through the hollow fibers, the continuous culture in the foregoing cell culture apparatus becomes possible, and stable collection of the highly concentrated useful substance can be carried out for a prolonged period.

It is noted that in the foregoing membrane separation device, it is desirable that only the low-molecular-weight substances be selectively removed. The molecular weight cutoff has to be at least within a range in which the high-molecular-weight useful substance does not permeate. For example, it is suitable that the range is between about 1000 and 200000, and preferably between about 3000 and 30000. In contrast, the molecular weight cutoff of the hollow fiber may be set to within a range in which the useful substance does not permeate. The range may be equal to or larger than the molecular weight cutoff of the membrane separation device.

Materials for the hollow fiber used in the apparatus of the present invention are not limited. However, examples thereof include an organic macromolecule, an inorganic porous body, and a metallic porous body. Also, the internal diameter of the hollow fiber may be between 10 and 1000 μm, and the membrane thickness may be between 2 and 500 μm. In particular, it is preferable that the internal diameter be between about 50 and 500 μm. Examples of the above organic macromolecular material include, for example, cellulose acetate, cellulose triacetate,

cellulose ester, polysulfone, polyolefin, polyfluorocarbon and polysiloxane.

It is preferable that the circulating flow rate in the culture-medium-circulating system be 1000 cm/min or less, and particularly 300 cm/min or less as a linear velocity. When the flow rate exceeds 1000 cm/min, the pressure inside the hollow fiber becomes too high. Accordingly, it is not preferable because the amount permeated into the cell culture space becomes large.

In contrast, it is preferable that the circulating flow rate of the produced-liquid-circulating system be between 0.1 and 1000 cm/min, and particularly between about 1 and 300 cm/min as a linear velocity. Consequently, when the flow rate is less than 0.1 cm/min, the replacement of liquid cannot be achieved, and the concentration of metabolites in the vicinity of the cells becomes too high, which inhibits the cell growth. When the flow rate exceeds 1000 cm/min, undesirable phenomena such as cell disruption and killing occur due to the affects of shearing force.

The transferred amount of the culture medium which permeates through the foregoing hollow fibers is made to be changed by controlling the pressure inside hollow fibers 1 by installing a chamber 7 in the culture-medium-circulating system as shown in, for example, Figure 1 (a drawing of an embodiment), by applying gas pressure to a culture medium 5 in this chamber 7, and by adjusting this pressure. Then, by controlling the liquid level of the culture medium 5 in the above chamber 7 and the liquid level of a culture medium (a produced liquid) in a chamber 19

which is installed in the produced-liquid-circulating system to each have a constant level, the flow of the culture medium in the cell culture apparatus becomes stable and the long-term continuous culture becomes possible.

Hereinafter, the present invention is described in detail by referring to embodiments. However, the following embodiments do not have a characteristic to limit the present invention. Addition of various design changes by pursuing the above- and below-described purport is included in the technical scope of the present invention.

[EMBODIMENTS]

Figure 1 is a schematic diagram showing an embodiment of the present invention.

A cell culture apparatus according to the present invention basically includes a cell culture device 2 and a membrane separation device 15. Next, the cell culture device 2 is configured such that a plurality of hollow fibers 1 having selective permeability are housed in a container 3. A cell culture space 4 is formed between the outer surface area of the hollow fibers 1 and the container 3. In the space 4, cells to be cultured are filled.

A culture medium 5 is supplied into the hollow fibers 1 by using a liquid-sending pump 6. The culture medium 5 is retained inside a chamber 7. A part of the culture medium 5 is made to permeate into the cell culture space 4 due to given driving force by applying pressure by an air-pressure-supplying device 8. The residual culture medium 5 which has not permeated into the cell culture space 4 passes through a line 20 to lead to a gas

exchanger 9. After the concentrations of oxygen and carbon dioxide are adjusted by this gas exchanger 9, a fresh culture medium 10 and a base 11 (e.g., 0.5 N NaOH) used for adjusting the optimal pH are added, and the mixture returns to the chamber 7 again (a culture-medium-circulating system). Also, partway along the line 20, a pH sensor 12a and a sensor 13a for sensing a dissolved oxygen content are installed, and by these sensors, the pH and dissolved oxygen content of the culture medium 5 which passes through the line 20 are measured.

In contrast, after temporary retained in a chamber 19 in a condition containing produced low-molecular-weight substances and a high-molecular-weight useful substance (hereinbelow referred to as a produced liquid 5a), the culture medium 5 in the cell culture space 4 is fed to a membrane separation device 15 by using a liquid-sending pump 14. Then, in the foregoing membrane separation device 15, low molecular metabolites such as lactic acid and an ammonium ion in the produced liquid 5a are separated and withdrawn to an outside system 17 via a separation membrane 16 (a hollow-fiber membrane in this embodiment) by using dynamic pressure produced by the liquid-sending pump 14. It is noted that the configuration of the separation membrane 16 is not limited to the hollow-fiber membrane shown, but may be a flat membrane that is usually used, a helical ultrafilter, or a reverse osmosis membrane.

The produced liquid 5a which has not permeated through the separation membrane 16 then returns to the cell culture space 4 again by passing through a line 21 (a produced-liquid-circulating system). This configuration is useful for suitably

stirring the cell culture space 4 and for uniformly dispersing the culture medium 5 (or the produced liquid 5a) or metabolites, etc., in the vicinity of the cells. Since the high-molecular-weight useful substance in the produced liquid 5a cannot permeate through the separation membrane 16, the useful substance is enriched by passing through the above-described produced-liquid-circulating system. This high-molecular-weight useful substance can be continuously withdrawn at a high concentration by collecting the produced liquid 5a or a certain flow rate by a liquid-sending pump 18 installed partway along the line 21. This configuration allows the environment in the cell culture space 4 to be uniformly maintained while the metabolites in the vicinity of the cells are appropriately removed, and enables the cells to be cultured at high density for a prolong period. Also, partway along the line 21, a pH sensor 12b and a sensor 13b for sensing a dissolved oxygen content are installed, and by these sensors, the pH and dissolved oxygen content of the produced liquid which passes through the line 21 are measured.

Besides, in the embodiment as illustrated in figure 1, although the pH sensors 12a and 12b and the sensors 13a and 13b for sensing dissolved oxygen are installed in both the culture-medium-circulating system and the produced-liquid-circulating system, the sensors may be installed in either of the systems.

In addition, in this embodiment, the hollow fibers 1 employ those made of cellulose acetate and having a molecular weight cutoff of 22,000, and the separation membrane 16 employs one

made of cellulose acetate and having a molecular cutoff of 5000, respectively.

In the meantime, in order to stabilize the flow of the culture medium 5 and the produced liquid 5a in the cell culture apparatus, liquid level meters 22a and 22b are installed in the chamber 7 of the culture-medium-circulating system and the chamber 19 of the produced-liquid-circulating system, respectively. The pressure inside the chamber 7 can be controlled by an air-pressure-supplying device 8 to keep the respective liquid levels of the chambers 7 and 19 constant. For example, by adjusting the amount of the culture medium permeated in such a manner, the flow rates can be balanced between the foregoing culture-medium-circulating system and the produced-liquid-circulating system. In addition, another air-pressure-supplying device 8 may be also installed in the chamber 19. Accordingly, a similar effect is achieved by adjusting the pressure in the chamber 19 as well as by adjusting the pressure in the chamber 7. It is noted that it is necessary to adjust the pressure in the chamber 7 to be higher than the pressure in the chamber 19 in such a case. Further, in order to achieve the object of the present invention, it is preferable that the foregoing liquid-sending pumps 6 and 14 employ synchronously operating pulsation. In addition, on taking the necessity to maintain a sterile condition for a prolonged period into consideration, as the foregoing liquid-sending pumps 6 and 14, it is most suitable to use a bellows pump which is superior in tightness.

Figure 2 is a schematic diagram of another embodiment of the present invention. The basic configuration is similar to the configuration as shown in Figure 1. The corresponding members denote the identical reference numerals, which avoids the overlapping description. This embodiment is one provided with a line 23 adapted to return a portion of the produced liquid 5a to be withdrawn from the membrane separation device 15 to the outside system 17, to the line 21. That is, among the low-molecular-weight substances separated by the membrane separation device 15, nutritional sources useful for cellular growth such as inorganic salts and amino acids are also included other than growth-inhibiting substances such as lactic acid and an ammonium ion. Accordingly, in order to utilize these nutritional sources as efficiently as possible, the configuration as shown in Figure 2 is adopted. It is needless to mention that the returned amount in this case should be a degree to which the above growth-inhibiting substances contained do not inhibit cellular growth.

From a similar viewpoint on the configuration as shown in Figure 2, for example, it is also possible to adopt the configuration as illustrated in Figure 3. Specifically, in the configuration as shown in Figure 3, the embodiment is provided with a line 24 which returns a portion of the produced liquid 5a to be withdrawn from the membrane separation device 15 to the outside system 17, to the line 20 of the culture-medium-circulating system. Accordingly, an effect similar to that for the configuration as shown in Figure 2 is achieved.

Figure 4 is a schematic diagram of a further additional embodiment of the present invention. In this embodiment, in

order to supplement nutritional sources including inorganic salts and amino acids, etc., which have been withdrawn to the outside system 17 together with the growth-inhibiting substances, an aqueous solution 25 containing inorganic salts and amino acids is separately prepared. This aqueous solution 25 is fed into the foregoing line 21 via a line 26.

[ADVANTAGES OF THE INVENTION]

As described above, according to the present invention, it is possible to keep the environment in the cell culture apparatus as uniform as possible by adopting the above-described configurations. The continuous culture of cells at high density becomes possible, and it also becomes possible to obtain a highly concentrated useful substance over a prolonged period.

4. BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1 to 4 are schematic diagrams showing various embodiments of the present invention.

1 ... Hollow fibers	2 ... Cell culture device
3 ... Container	4 ... Cell culture space
5 ... Culture medium	5a ... Produced liquid
6, 14, 18 ... Liquid-sending pump	
7, 19 ... Chamber	8 ... Air-pressure-supplying device
9 ... Gas exchanger	15 ... Membrane separation device

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